

CLAIMS

1. A method for selecting a strain of a unicellular, aquatic microorganism capable of heterotrophic growth and capable of producing omega-3 fatty acids comprising the steps of:
 - a. selecting candidate microorganisms of a size between about $1\mu\text{m}$ and $25\mu\text{m}$; then
 - b. culturing a sample containing said size-selected microorganism in a medium comprising a source of organic carbon, assimilable nitrogen, assimilable phosphorous, and a microbial growth factor under heterotrophic conditions to produce single colony isolates of colorless or lightly pigmented cells; and
 - c. testing for the presence of omega-3 fatty acids produced by the single colony isolates, whereby a strain of a unicellular organism capable of producing omega-3 fatty acids is selected.
2. A method according to claim 1 wherein the organisms are collected from naturally occurring saline or brackish waters.

3. A method according to claim 1 wherein the candidate microorganisms are incubated at 30°C for 24 hours prior to culturing.

4. A method according to claim 1 wherein the medium comprises an antibiotic to inhibit bacterial growth.

5. A method according to claim 1 wherein a microbial growth factor is provided by yeast extract or corn steep liquor.

6. A strain of unicellular microorganism capable of heterotrophic growth and capable of producing omega-3 fatty acids selected by the method of claim 1.

7. A strain of unicellular microorganism of the order Thraustochytriales and family Thraustochytriaceae selected by the method of claim 1.

8. A strain of unicellular microorganism of the order Thraustochytriales and family Thraustochytriaceae and the genus Thraustochytrium or genus Schizochytrium selected by the method of claim 1.

9. A unicellular, aquatic microorganism of the order Thraustochytriales at least a portion of whose cells range in diameter from about 1.0 μm to about 25 μm , having the capacity for sustained heterotrophic growth

in a medium comprising organic carbon, assimilable nitrogen, assimilable phosphorous and a microbial growth factor, and capable of producing omega-3 fatty acids, wherein at least 7.8 percent by weight of total fatty acids is C20:5w3.

10. A unicellular microorganism according to claim 9 wherein at least 10% by weight of total fatty acids is C20:5w3.

11. A unicellular microorganism according to claim 9 wherein at least 68% by weight of total fatty acids is omega-3 fatty acids.

12. A unicellular microorganism according to claim 11 comprising Thraustochytrium strain 23B (ATCC number 20892).

13. A unicellular microorganism according to claim 9 wherein not more than 10% by weight of total fatty acids is omega-6 fatty acids.

14. A unicellular microorganism according to claim 13 comprising Thraustochytrium strain 12B (ATCC number 20890).

15. A unicellular microorganism according to claim 10 wherein at least 68% by weight of total fatty acids is omega-3 fatty acids.

16. A unicellular microorganism according to claim 11 wherein at least 75% by weight of total fatty acid is omega-3 fatty acids.

5 17. A unicellular microorganism according to ~~claim 13~~ wherein at least 10% by weight of total fatty acids is C20:5w3.

18. A unicellular microorganism according to claim 10 wherein not more than 5% by weight of total fatty acids is omega-6 fatty acids.

10 19. A unicellular microorganism according to claim 11 wherein not more than 10% by weight of total fatty acids is omega-6 fatty acids.

15 20. A unicellular microorganism according to claim 15 wherein not more than 10% by weight of total fatty acids is omega-6 fatty acids.

21. A unicellular microorganism according to ~~claim 18~~ wherein at least 68% by weight of total fatty acids is omega-3 fatty acids.

22. A unicellular microorganism according to claim 11 wherein not more than 5% by weight of total fatty acids is omega-6 fatty acids.

23. A unicellular microorganism according to claim 22 wherein not more than 2% by weight of total fatty acids is omega-6 fatty acids.

101
24. A unicellular, aquatic microorganism of the order Thraustochytriales, at least a portion of whose cells range in diameter from about 1.0 μm to about 25 μm , having the capacity for sustained heterotrophic growth in a medium comprising organic carbon, assimilable nitrogen, assimilable phosphorous and a microbial growth factor, and capable of producing omega-3 fatty acids, wherein not more than 10% by weight of total fatty acids is omega-6 fatty acids.

25. A unicellular microorganism according to claim 24 wherein not more than 5% by weight of total fatty acids is omega-6 fatty acids.

26. A unicellular microorganism according to claim 24 wherein not more than 2% by weight of total fatty acids is omega-6 fatty acids.

27. A unicellular, aquatic microorganism of the order Thraustochytriales, at least a portion of whose diameter

from about 1.0 μm to about 25 μm , having the capacity for sustained heterotrophic growth in a medium comprising organic carbon, assimilable nitrogen, assimilable phosphorous and a microbial growth factor, and capable of producing omega-3 fatty acids, wherein at least 68% by weight of total fatty acids is omega-3 fatty acids.

28. A unicellular microorganism according to claim 27 wherein at least 75% of total fatty acids is omega-3 fatty acids.

29. A unicellular, aquatic microorganism of the order Thraustochytriales, at least a portion of whose cells range in diameter from about 1.0 μm to about 25 μm , having the capacity for sustained heterotrophic growth in a medium comprising organic carbon, assimilable nitrogen, assimilable phosphorous and a microbial growth factor, and capable of producing at least 25% of total fatty acids as omega-6 fatty acids.

30. A unicellular, aquatic microorganism of the order Thraustochytriales, at least a portion of whose cells range in diameter from about 1.0 μm to about 25 μm , having the capacity for sustained heterotrophic growth in a medium comprising organic carbon, assimilable nitrogen, assimilable phosphorous and a microbial growth factor, and capable of producing omega-3 fatty acids,

wherein at least 94% by weight of said omega-3 fatty acids is C22:6w3.

31. A unicellular microorganism according to claim 30 wherein at least 3% by weight of said omega-3 fatty acids is C20:5w3.

32. A unicellular microorganism according to claim 31 comprising Schizochytrium strain S8 (ATCC No. 20889).

33. A unicellular, aquatic microorganism of the order Thraustochytriales, at least a portion of whose diameter from about 1.0 μm to about 25 μm , having the capacity for sustained heterotrophic growth in a medium comprising organic carbon, assimilable nitrogen, assimilable phosphorous and a microbial growth factor, and capable of producing omega-3 fatty acids, wherein said organism is capable of growing at the rate of at least 5.5 doublings of cell number per day at 25°C in M-5 medium.

34. A unicellular microorganism according to claim 33 comprising Schizochytrium strain S31 (ATCC No. 20888) or Schizochytrium strain S8 (ATCC No. 20889).

35. A method for the heterotrophic culture of a unicellular, aquatic microbial strain of the order Thraustochytriales capable of producing omega-3 fatty acids, comprising

no recovery
step

culturing the strain in a medium comprising a source of organic carbon, a source of assimilable nitrogen and a microbial growth factor.

- 5 36. The method of claim 35 comprising the added step of culturing the cells in a medium having reduced assimilable nitrogen to enhance total lipid and omega-3 fatty acid levels.
37. The method of claim 35 wherein the medium comprises at least 0.88 grams per liter of Na.
- 10 38. The method of claim 35 wherein the medium comprises not greater than 2.15 g/l of Na.
39. The method of claim 35 wherein the medium comprises corn steep liquor or yeast extract.
- 15 40. The method of claim 35 wherein the medium comprises assimilable phosphorous equivalent to at least 0.2 grams per liter of KH_2PO_4 .
41. The method of claim 35 wherein the culturing temperature is greater than or equal to 15°C.
- 20 42. The method of claim 35 wherein the culturing temperature is greater than or equal to 30°C.

43. The method of claim 35 wherein the pH is controlled from 5.5 to 7.5.
44. The method of claim 35 wherein the aquatic microbial strain is a unicellular, aquatic microorganism of the order Thraustochytriales and family Thraustochytriaceae.
45. A method according to claim 35 wherein the aquatic microbial strain is selected from the genus Thraustochytrium or the genus Schizochytrium.
46. A method according to claim 35 wherein the aquatic microbial strain is selected from the group consisting of Thraustochytrium U42-2 (ATCC No. 20891), Schizochytrium S31 (ATCC No. 20888), Schizochytrium S8 (ATCC No. 20889), Thraustochytrium 12B (ATCC No. 20890), Thraustochytrium 23B (ATCC No. 20892), Thraustochytrium aureum (ATCC No. 28211), Thraustochytrium aureum (ATCC No. 34304), Thraustochytrium roseum (ATCC No. 28210), Thraustochytrium striatum (ATCC No. 24473) and Schizochytrium aggregatum (ATCC No. 28209).
47. The method of claim 35 wherein carbon and nitrogen are supplied by ground, hydrolyzed or unhydrolyzed grain.
48. The method of claim 35 wherein the growth medium is supplemented with extracted (lipids removed) biomass of cells of the genus Thraustochytrium.

49. The method of claim 35 wherein the microorganism belongs to the genus Thraustochytrium and carbon and nitrogen are supplied by ground, unhydrolyzed grain.

50. A method of making a microbial product enriched in omega-3 fatty acid content comprising:

a. harvesting cells of a microorganism at least a portion of whose cells range in diameter from about 1.0 μm to about 25 μm of the order Thraustochytriales having the capacity for sustained heterotrophic growth in a medium comprising organic carbon, assimilable nitrogen, assimilable phosphorous and a microbial growth factor and capable of omega-3 fatty acid production;

b. concentrating and preserving the harvested cells under conditions suitable for maintaining stability of omega-3 fatty acids

51. The method of claim 50 wherein the microorganism is essentially white or colorless when grown under the conditions employed for omega-3 fatty acid production.

52. The method of claim 50 comprising the additional step of culturing the cells under conditions of nitrogen

limitation in order to enhance omega-3 fatty acid production.

53. The method of claim 50 comprising the additional step of culturing the cells under conditions of assimilable phosphorous concentration equivalent to at least 0.2 grams per liter KH_2PO_4 .

54. The method of ~~claim 50~~ comprising the additional step of culturing the cells wherein the pH is controlled from 5.5 to 7.5.

55. The method of claim 50 wherein the microorganism is selected from the genus Thraustochytrium or the genus Schizochytrium.

56. The method of claim 50 wherein the microorganism is selected from the group consisting of Thraustochytrium U42-2 (ATCC No. 20891), Schizochytrium S31 (ATCC No. 20888), Schizochytrium S8 (ATCC No. 20889), Thraustochytrium 12B (ATCC No. 20890), Thraustochytrium 23B (ATCC No. 20892), Thraustochytrium aureum (ATCC No. 28211), Thraustochytrium aureum (ATCC No. 34304), Thraustochytrium roseum (ATCC No. 28210), Thraustochytrium striatum (ATCC No. 24473) and Schizochytrium aggregatum (ATCC No. 28209).

57. The method of claim 50 wherein the cells are concentrated and preserved by lyophilization.

58. The method of claim 50 wherein the cells are concentrated and preserved by adding an antioxidant.

59. The method of claim 50 wherein the cells are concentrated and preserved by sealing under an inert atmosphere.

60. The method of claim 50 wherein the cells are concentrated and preserved by refrigeration or freezing.

61. A method of making omega-3 fatty acids comprising culturing cells of a microorganism at least a portion of whose cells range in diameter from about 1.0 μm to about 25 μm of the order Thryaustochytridales having the capacity for sustained heterotrophic growth in a growth medium comprising organic carbon, assimilable nitrogen, assimilable phosphorous and growth factors, and capable of omega-3 fatty acid production, harvesting the cells from the culture medium, and extracting omega-3 fatty acids from the harvested cells.

62. A food additive or nutritional supplement for humans comprising refined oils of claim 61.

63. The method of claim 61 wherein the microorganism is selected from the genus Thraustochytrium or the genus Schizochytrium.

5 64. The method of claim 61 wherein the microorganism is selected from the group consisting of Thraustochytrium U42-2 (ATCC No. 20891), Schizochytrium S31 (ATCC No. 20888), Schizochytrium S8 (ATCC No. 20889), Thraustochytrium 12B (ATCC No. 20890), Thraustochytrium 23B (ATCC No. 20892), Thraustochytrium aureum (ATCC No. 28211), Thraustochytrium aureum (ATCC No. 34304), Thraustochytrium roseum (ATCC No. 28210), Thraustochytrium striatum (ATCC No. 24473) and Schizochytrium aggregatum (ATCC No. 28209).

10

15 65. The method of claim 61 wherein the organic carbon is a carbohydrate.

66. The method of claim 61 wherein the medium has a sodium concentration of at least 0.88 g/l.

67. The method of claim 61 wherein the medium comprises not greater than 2.15 grams per liter of Na.

20 68. The method of claim 61 wherein the cells are cultured at a temperature greater than or equal to 15°C.

69. The method of claim 61 wherein the cells are cultured at a temperature greater than or equal to 30°C.
70. The method of claim 61 wherein the cells are cultured under conditions of assimilable phosphorous concentration equivalent to at least 0.2 grams per liter KH_2PO_4 .
71. The method of claim 61 wherein the cells are cultured under conditions of pH controlled from 5.5 to 7.5.
72. The method of claim 61 wherein, prior to harvesting, the cells are cultured in a medium lacking assimilable nitrogen and incubated therein.
73. The method of claim 61 wherein the omega-3 fatty acids are extracted by solvent extraction.
74. The method of claim 61 wherein the omega-3 fatty acids are extracted by supercritical fluid fractionation.
75. The method of claim 61 wherein the harvested cells are broken or lysed and the omega-3 fatty acids are extracted into vegetable or other edible oil.
76. The method of claim 61 wherein the microorganism contains less than 5% by weight of total fatty acids as omega-6 highly unsaturated fatty acids and the omega-3

highly unsaturated fatty acids are purified by the steps of:

- (a) rupturing the harvested cells to yield ruptured cells;
- (b) solvent extracting lipid mixture from the ruptured cells;
- (c) hydrolyzing the lipid mixture;
- (d) removing non-saponifiable compounds from the product of step (c) by solvent extraction; and
- (e) cold-crystallizing the product of step (d) to yield purified omega-3 highly unsaturated fatty acids, or a mixture of purified highly unsaturated fatty acids.

77. A fatty acid mixture containing at least 90% by weight omega-3 highly unsaturated fatty acids, prepared by the method of claim 61 wherein the microorganism contains less than 5% by weight of total fatty acids as omega-6 highly unsaturated fatty acids and the omega-3 highly unsaturated fatty acids are purified by the steps of:

- (a) rupturing the harvested cells to yield ruptured cells;

(b) solvent extracting lipid mixture from the ruptured cells;

(c) hydrolyzing the lipid mixture;

(d) removing non-saponifiable compounds from the product of step (c) by solvent extraction; and

(e) cold-crystallizing the product of step (d) to yield purified omega-3 highly unsaturated fatty acids, or a mixture of purified highly unsaturated fatty acids.

78. A food additive or nutritional supplement for humans comprising the fatty acid mixture of claim 61, wherein the microorganism contains less than 5% by weight of total fatty acids as omega-6 highly unsaturated fatty acids and the omega-3 highly unsaturated fatty acids are purified by the steps of:

(a) rupturing the harvested cells to yield ruptured cells;

(b) solvent extracting lipid mixture from the ruptured cells;

(c) hydrolyzing the lipid mixture;

(d) removing non-saponifiable compounds from the product of step (c) by solvent extraction; and

(e) cold-crystallizing the product of step (d) to yield purified omega-3 highly unsaturated fatty acids, or a mixture of purified highly unsaturated fatty acids.

79. A microbial product enriched in omega-3 fatty acid content comprising cellular biomass of a unicellular aquatic microorganism of the order Thraustochytriales at least a portion of whose cells range in diameter from about 1.0 μm to about 25 μm , having the capacity for sustained heterotrophic growth in a medium comprising organic carbon, assimilable nitrogen, assimilable phosphorous and a microbial growth factor, and capable of producing omega-3 highly unsaturated fatty acids, wherein at least 7.8 percent by weight of total fatty acids is C20:5w3.

80. A microbial product according to claim 79 wherein at least 10% by weight of total fatty acids is C20:5w3.

81. A microbial product according to claim 79 wherein at least 68% by weight of total fatty acids is omega-3 highly unsaturated fatty acids.

82. A microbial product according to claim 81 comprising Thraustochytrium strain 23B (ATCC number 20892).

83. A microbial product according to claim 79 wherein not more than 10% by weight of total fatty acids is omega-6 highly unsaturated fatty acids.

84. A microbial product according to claim 83 comprising Thraustochytrium strain 12B (ATCC number 20890).

85. A microbial product according to claim 80 wherein at least 68% by weight of total fatty acids is omega-3 highly unsaturated fatty acids.

86. A microbial product according to claim 81 wherein at least 75% by weight of total fatty acid is omega-3 fatty acids.

87. A microbial product according to claim 83 wherein at least 10% by weight of total fatty acids is C20:5w3.

88. A microbial product according to claim 80 wherein not more than 5% by weight of total fatty acids is omega-6 highly unsaturated fatty acids.

89. A microbial product according to claim 81 wherein not more than 10% by weight of total fatty acids is omega-6 highly unsaturated fatty acids.

90. A microbial product according to claim 85 wherein not more than 10% by weight of total fatty acids is omega-6 highly unsaturated fatty acids.

5 91. A microbial product according to claim 88 wherein at least 68% by weight of total fatty acids is omega-3 highly unsaturated fatty acids.

92. A microbial product according to claim 81 wherein not more than 5% by weight of total fatty acids is omega-6 highly unsaturated fatty acids.

10 93. A microbial product according to claim 92 wherein not more than 2% by weight of total fatty acids is omega-6 highly unsaturated fatty acids.

15 94. A microbial product enriched in omega-3 highly unsaturated fatty acid content comprising cellular biomass of a unicellular, aquatic microorganism of the order Thraustochytriales at least a portion of whose cells range in diameter from about 1.0 μm to about 25 μm , having the capacity for sustained heterotrophic growth in a medium comprising organic carbon, 20 assimilable nitrogen, assimilable phosphorous and a microbial growth factor, and capable of producing omega-3 highly unsaturated fatty acids, wherein not more than

10% by weight of total fatty acids is omega-6 highly unsaturated fatty acids.

95. A microbial product according to claim 94 wherein not more than 5% by weight of total fatty acids is omega-6 highly unsaturated fatty acids.

96. A microbial product according to claim 94 wherein not more than 2% by weight of total fatty acids is omega-6 highly unsaturated fatty acids.

97. A microbial product enriched in omega-3 highly unsaturated fatty acid content comprising cellular biomass of a unicellular aquatic microorganism of the order Thraustochytriales, at least a portion of whose diameter from about 1.0 μm to about 25 μm , having the capacity for sustained heterotrophic growth in a medium comprising organic carbon, assimilable nitrogen, assimilable phosphorous and a microbial growth factor, and capable of producing omega-3 highly unsaturated fatty acids, wherein at least 68% by weight of total fatty acids is omega-3 highly unsaturated fatty acids.

98. A microbial product according to claim 97 wherein at least 75% of total fatty acids is omega-3 highly unsaturated fatty acids.

99. A microbial product enriched in omega-3 highly unsaturated fatty acids content comprising cellular biomass of a unicellular aquatic microorganism of the order Thraustochytriales, at least a portion of whose cells range in diameter from about 1.0 μm to about 25 μm , having the capacity for sustained heterotrophic growth in a medium comprising organic carbon, assimilable nitrogen, assimilable phosphorous and a microbial growth factor, and capable of producing omega-3 highly unsaturated fatty acids, wherein at least 94% by weight of said omega-3 highly unsaturated fatty acids is C22:6w3.

100. A microbial produce according to claim 99 wherein at least 3% by weight of said omega-3 highly unsaturated fatty acids is C20:5w3.

101. A microbial product according to claim 100 wherein the microorganism is Schizochytrium strain S8 (ATCC No. 20889).

102. A microbial product enriched in omega-3 highly unsaturated fatty acid content comprising cellular biomass of a unicellular aquatic microorganism of the order Thraustochytriales, at least a portion of whose diameter from about 1.0 μm to about 25 μm , having the capacity for sustained heterotrophic growth in a medium comprising organic carbon, assimilable nitrogen,

assimilable phosphorous and a microbial growth factor,
and capable of producing omega-3 highly unsaturated
fatty acids, wherein said organism is capable of growing
at the rate of at least 5.5 doublings of cell number per
day at 25°C in M-5 medium.

103. A microbial product according to claim 102 wherein the
microorganism comprises Schizochytrium strain S31 (ATCC
No. 20888) or Schizochytrium strain S8 (ATCC No. 20889).

104. An animal feed ingredient or feed product comprising
biomass of cells of the order Thraustochytriales or
omega-3 highly unsaturated fatty acids purified
therefrom.

105. An animal feed ingredient comprising a microbial product
according to claim 79.

106. An animal feed product comprising an animal feed
ingredient according to claim 105.

107. An animal feed ingredient comprising extracted highly
unsaturated fatty acids of a microbial product according
to claim 79.

108. An animal feed ingredient comprising a microbial product
according to claim 94.

109. An animal feed product comprising an animal feed ingredient according to claim 108.

110. An animal feed ingredient comprising extracted highly unsaturated fatty acids of a microbial product according to claim 94.

111. An animal feed ingredient comprising a microbial product according to claim 97.

112. An animal feed product comprising an animal feed ingredient according to claim 111.

113. An animal feed ingredient comprising extracted highly unsaturated fatty acids of a microbial product according to claim 97.

114. An animal feed ingredient comprising a microbial product according to claim 99.

115. An animal feed product comprising an animal feed ingredient according to claim 114.

116. An animal feed ingredient comprising extracted highly unsaturated fatty acids of a microbial product according to claim 99.

117. An animal feed ingredient comprising a microbial product according to claim 102.

118. An animal feed product comprising an animal feed ingredient according to claim 117.

5 119. An animal feed ingredient comprising extracted highly unsaturated fatty acids of a microbial product according to claim 102.

10 120. A method of producing poultry eggs enriched in omega-3 highly unsaturated fatty acid content comprising feeding poultry a poultry feed supplemented with a microbial product comprising biomass of cells of the order Thraustochytriales or omega-3 highly unsaturated fatty acids purified therefrom.

Same as
126

15 121. A method of producing poultry eggs enriched in omega-3 highly unsaturated fatty acid content comprising feeding poultry a poultry feed supplemented with microbial product according to claim 79.

20 122. A method of producing poultry eggs enriched in omega-3 highly unsaturated fatty acid content comprising feeding poultry a poultry feed supplemented with microbial product according to claim 94.

123. A method of producing poultry eggs enriched in omega-3 highly unsaturated fatty acid content comprising feeding poultry a poultry feed supplemented with microbial product according to claim 97.

5 124. A method of producing poultry eggs enriched in omega-3 highly unsaturated fatty acid content comprising feeding poultry a poultry feed supplemented with microbial product according to claim 99.

10 125. A method of producing poultry eggs enriched in omega-3 highly unsaturated fatty acid content comprising feeding poultry a poultry feed supplemented with microbial product according to claim 102.

15 126. A method of producing poultry eggs enriched in omega-3 highly unsaturated fatty acid content comprising feeding poultry a poultry feed supplemented with a microbial product comprising biomass of cells of the order Thraustochytriales or omega-3 highly unsaturated fatty acids purified therefrom.

20 127. An animal product enriched in omega-3 highly unsaturated fatty acid content for human consumption comprising flesh, or eggs of an animal fed with feed comprising the animal feed ingredient of claim 105.

128. An animal product enriched in omega-3 highly unsaturated fatty acid content for human consumption comprising flesh, or eggs of an animal fed with feed comprising the animal feed ingredient of claim 108.

5 129. An animal product enriched in omega-3 highly unsaturated fatty acid content for human consumption comprising flesh, or eggs of an animal fed with feed comprising the animal feed ingredient of claim 111.

10 130. An animal product enriched in omega-3 highly unsaturated fatty acid content for human consumption comprising flesh, or eggs of an animal fed with feed comprising the animal feed ingredient of claim 114.

15 131. An animal product enriched in omega-3 highly unsaturated fatty acid content for human consumption comprising flesh, or eggs of an animal fed with feed comprising the animal feed ingredient of claim 117.